COMPARISON OF SUPERCRITICAL FLUID EXTRACTION AND HYDRODISTILLATION METHOD FOR DETERMINATION OF AGARWOOD ESSENTIAL OIL

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ABSTRACT

Gaharu or agarwood is a tree in the family *Thymelaeceae*. Gaharu is the occasional product of two to four spesies in the family *Thymelaeaceae*. Mature trees will grow up to 40 meter in height and 40 centimeter in diameter. Gaharu trees in natural forests began to produce agarwood resin at the age of 20 to 45 years, depending on the resistance of trees and tree injury response. It is also one of the most expensive natural products existing today. Gaharu is many used in perfumes, medicines, and toiletery product. The objective of the experiment is to identify the compounds of the gaharu using supercritical fluid extraction (SFE) and hydrodistillation extraction methods. Results obtained from the different instrument method were then compared to analyze the compounds. The essentials oil of gaharu was obtained by supercritical extraction of components from solid materials. This is a relatively new process. From previous experiment this process gives a better quality extract but the capital costs are high. Carbon dioxide is usually used for solvent in this process. Then, a common conventional method used to extract the essential oil from gaharu is hydrodistillation. The problems of this technique are low efficiency and acquire high and continuous heating and required long extraction time. The essential oil was extract will be analyzed by gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The results from GC-MS and GS-FID were compared to produce composition of compound from gaharu with different method of extractions.

ABSTRAK

Gaharu terdiri daripada pokok di dalam keluarga Thymelaeceae. Kadang-kadang terdapat dua hingga empat jenis spesies produk gaharu dalam keluarga Thymelaeaceae. Pokok dewasa akan tumbuh hingga 40 meter tingginya dan 40 sentimeter untuk diameternya. Pokok gaharu dalam hutan semulajadi mula menghasilkan gaharu resin pada usia 20 hingga 45 tahun bergantung pada ketahanan pokok dan tindakbalas kecederaan pokok. Ia juga salah satu bahan semulajadi yang mahal pada ketika ini. Gaharu banyak digunakan dalam produk minyak wangi, perubatan dan alatan tandas. Tujuan kajian ini adalah untuk menentukan komponen gaharu dengan menggunakan ekstraksi bendalir kritikal dan kaedah destilasi air. Dengan menggunakan kedua-dua alatan ini, perbezaan keputusan dapat dibandingkan dalam menanalisis sebatian. Pati minyak gaharu dapat dihasilkan melalui komponen interaksi bendalir kritikal indeks daripada bahan pepejal. Ini adalah proses baru. Daripada kajian yang lepas, proses ini memberi ekstrak kualiti yang baik tetapi memerlukan cos yang tinggi. Dalam proses ini, karbon dioksida biasanya digunakan sebagai bahan pelarut. Kemudian, kaedah lama digunakan untuk ekstrak pati minyak daripada gaharu ialah destilasi air. Masalah daripada teknik ini ialah rendah dalam kecekapannya, sentiasa dipanaskan dan memerlukan masa pengekstrakan yang lama. Pati minyak yang sudah diekstrak akan dianalaisis dengan menggunakan GC-FID dan GC-MS. Keputusan sebatian daripada GC-FID dan GC-MS daripada komposisi sebatian gaharu dapat dibandingkan dengan kaedah pengekstrakan yang berbeza.

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LIST OF SYMBOLS

α	Alpha
β	Beta
°C	Celsius
δ	Delta
γ	Gamma
μ	Micro
%	Percentage

LIST OF ABBREVIATIONS

b.p	Boiling Point
CAS	Chemical Abstracts Service
dbh	Diameter at breast height
GC	Gas chromatography
GC-FID	Gas Chromatography-Flame Ionization Detector
GC-MS	Gas Chromatography-Mass Spectrometry
HD	Hydrodistillation
KI	Kovats Index
Min	Minutes
NIST	National Institute of Standards Technology
Psi	Pound per square inch
RT	Retention time
Ref	References
SFE	Supercritical Fluid Extraction

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Gaharu is the resinous, fragrant and highly valuable heartwood produced by *Aquilaria malaccensis* and other species of the Indomalesian tree genus *Aquilaria*, from the family of Thymelaeceae. There are fifteen species in the *Aquilaria g*enus and eight are known to produce gaharu. In Malaysia, gaharu is primarily produced from *A. Malaccensis*, *A .Hirta*, *A.Microcarpa*, *A.Rostrata* and *A.Beccariana* (Chang *et al*., 2002) and they are large evergreen trees growing over 15-30 m tall and 1.5-2.5 m in diameter, and has white flowers (Chakrabarty *et al*., 1994).

In this study, the gaharu chipwood used to produce essential oil and obtained from different instrument which is by using hydro distillation (HD) and supercritical fluid extraction (SFE) method. Gaharu essential oil is highly prized for the scent produced and the oil is widely used in industries. Generally, gaharu oils are mixture of sesquiterpenes, sesquiterpene alcohols, oxygenated compounds, chromone derivatives and resin (Chang *et al.,* 2002). Plant extracts as seen as a way of meeting the demanding requirement of the modern industry for the past two decades (Simandi *et al.,* 1996).

Besides that, hydrodistillation (HD) method is different with supercritical fluid extraction (SFE) which is created by heating any substance above its critical temperature and raising its pressure above its critical limit as well. Critical temperature refers to the highest temperature at which a gas can be converted to a liquid through an increase in pressure. Similarly, critical pressure is the highest pressure a liquid can be converted to a gas by increasing in temperature. Parameters such as the density, diffusivity and viscosity of SFE are therefore intermediary of liquids and gases.

The aim of the present work is to investigate of the effect of different parameters, such as pressure, temperature, modifier volume and dynamic and static extraction time on the supercritical fluid extraction of *agarwood* (gaharu). The essential oil obtained by hydrodistillation was used for comparison. These extraction methods will be further discussed in the literature review.

1.2 Problem statement

The main problem in this research is lack information of gaharu and its essential oil. Other than that, understanding operation of different instruments is important to determine essential oil from gaharu. Nowadays, the most popular method to extract gaharu essential oil is the traditional hydrodistillation method. The efficiency of this method is relatively low and it is too time consuming. All of this will result in higher operating cost because of the process is not efficient in cost and processing period time is much longer. Furthermore, prolong action of hot water can cause hydrolysis of some constituents of the essential oils such as ester, (Mohammad, 2008).

Another problem is current method also including the extraction using solvent. Even though it take shorter time than the hydrodistillation, the oil produced by this method is not suitable for skin use (Wilson, 1995). Besides that, traditional method different with a supercritical fluid extraction (SFE) method which is large number of compounds that can be used as a fluid in supercritical techniques, but by far the most widely used is carbon dioxide. The solubility of polar compounds and the selectivity of the process can be increased by adding small quantities of other solvents, such as ethanol, in the fluid that named as co-solvent or modifier.

The oils isolated under various SFE and HD conditions were analyzed by GC-FID and GC-MS. Sensory analysis was used to determine the optimum oil composition that was compared with that of essential oil isolated by hydro distillation. The problem is when analyzed their chemical compound by GC-FID and GC-MS,that do not provide the full identification of the components and consequently do not give a guarantee of authenticity.

1.3 Objectives

The main objectives of this research are:

1. To compare extraction of essential oil from gaharu using different method namely supercritical fluid (SFE) method and hydro distillation (HD) method.

2. To identify the chemical compound present in the essential oil of gaharu using Gas Chromatography -Flame Ionization Detector (GC-FID) Gas Chromatography-Mass Spectrometer (GC-MS).

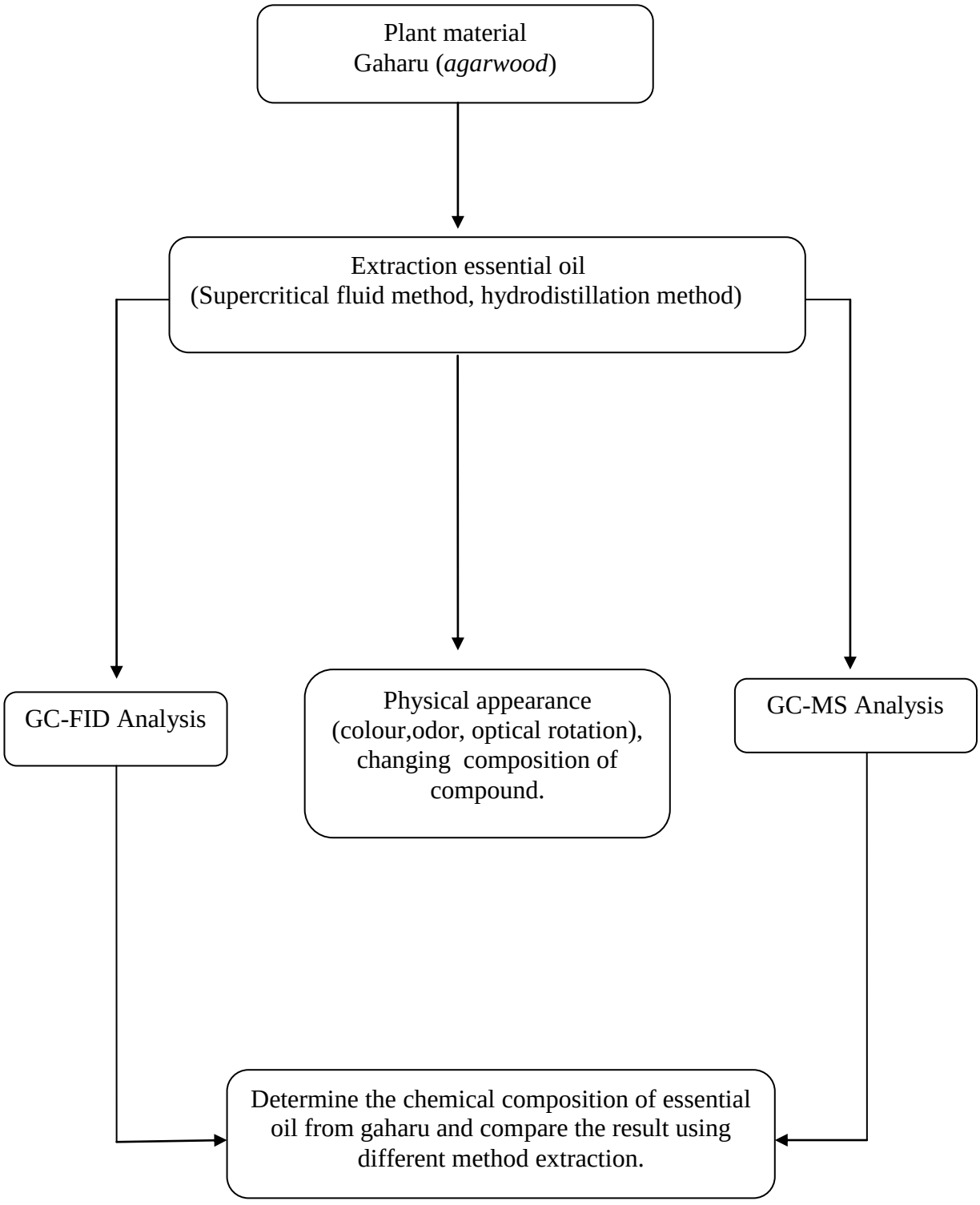

Figure 1.1: The flow chart research of analysis essential oil from gaharu using different method extraction.

1.4 Scope of study

The scope of this study is to compare essential oil from gaharu between two method by using supercritical fluid extraction (SFE) and hydro distillation (HD) . In order to achieve the objective the scope of study is about the effect of different instrument on essential oil from gaharu and influent result to determine composition essential oil using gas chromatography (GC-FID and GC-MS).

CHAPTER 2

LITERATURE REVIEW

2.1 Background of Aloeswood (Gaharu)

In Malaysia, the tree of *Aquilaria* is called as karas and its fragrant wood is known as gaharu. Five species of *Aquilaria* are recorded in Peninsular of Malaysia and all are believed to be able to produce oleoresins. The most popular species generally associated with gaharu is *A. Malaccensis* (Chang *et al.*, 2002). The grade of gaharu essential oil are divided by five types, which are Grade Super A, A, B, C, and D. The Grade Super A is the most expensive compared to the other grades. The grade (and hence value) of agarwood and agarwood derivatives such as oil is determined by a complex set of factors including country of origin, fragrance strength and longevity, wood density, product purity, resin content, colour, and size of the form traded.

Aquilaria spp. (Thymelaeaceae) are the principal source of Gaharu (Soehartono and Newton, 2001), a resin-impregnated heartwood that is fragrant and highly valuable. Other names used by both collectors and traders of the fragrant wood are agar, aloeswood, eaglewood, kalambak or gaharu depending on the country and generally encompass the fragrant wood produced by most species of *Aquilaria* (Ng *et al.,* 1997). Agarwood is a fast growing, evergreen tree, that normally grows to 18-21 m but sometimes up to 40 m in height. The trees occasionally become infected with a parasite mould and begin to produce an aromatic resin in response to this attack. As the fungus grows, the tree produces a very rich, dark resin within the heartwood. The resin is commonly called Jinko, Aloeswood, Agarwood or Oud and is valued in many cultures for its distinctive fragrance, thus it is used for incense and perfumes (Fauzi, 2008).

Gaharu is a resinous wood that sometimes occurs in trees belong to the *Aquilaria* genus, *Thymelaeceae* family. Gaharu producing species are found from India eastwards throughout Southeast Asia (Indonesia, Thailand, Cambodia, Laos, Vietnam, and Malaysia). *Aquilaria* is a fast-growing, archaic tropical forest tree. There are different names for gaharu such as ch'en hsiang, eagle wood, jin-koh, oud and others. There are 15 species of *Aquilaria*. In Malaysia, there are five species of *Aquilaria* which are *Aquilaria Malaccensis, Aquilaria Microcorpa, Aquilaria HIrta, Aquilaria Rostrata* and *Aquilaria Becanana.* Agarwood contains more than 12 chemical components that can be extracted.

Figure 2.1: Aloeswood (gaharu).

(Source: Mohd Rosli Bin Ramly, 2006)

The use of gaharu for perfumery extends back several thousands of years, and is referenced, for example, in the Old Testament several times using the term ‘aloes’. Both gaharu smoke and oil are customarily used as perfume in the Middle East (Chakrabarty et al, 1994). In India, various grades of gaharu are distilled separately before blending to produce final ‘attar’. Minyak attar is a water based perfume containing gaharu oil, which is traditionally used by Muslims to lace prayer clothes (Yaacob, 1999).

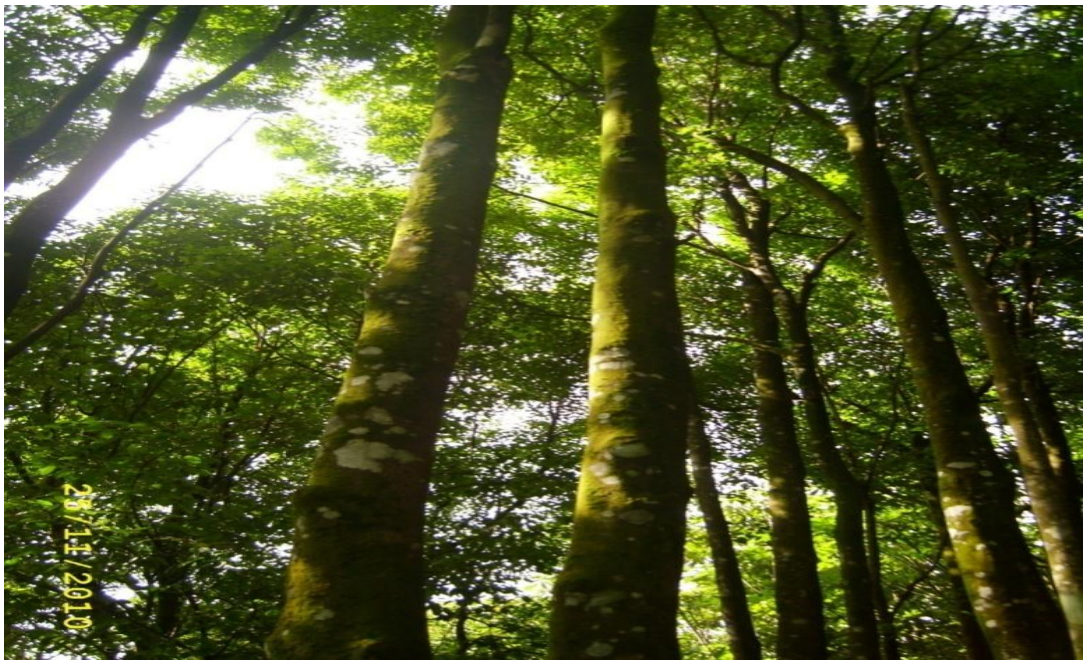

Figure 2.2: Tree of gaharu

2.1.1 Gaharu Species

Three species of *Aquilaria* are found in Malaysia: *A. hirta*, *A. malaccensis* and *A. rostrata*. *Aquilaria malaccensis* is well distributed throughout Peninsular Malaysia, except for the States of Kedah and Perlis. It is confined mainly to plains, hill slopes and ridges up to 750 m in both primary and secondary Malaysian lowland and hill dipterocarp forests (Jantan, 1990). The average diameter growth rate of *A. malaccensis* in native forests in Malaysia is rather low, e.g. a mean of 0.33 cm/ year, but the fastest-growing larger specimens are reported to grow at 0.8-1 cm/year (La Frankie, 1994). Although *A. malaccensis* enjoys good geographical coverage, its occurrence is rather rare.

The following species of *Aquilaria* produce agarwood:

1. AQUILARIA SUBINTEGRA, found in Thailand
2. AQUILARIA CRASSNA, found in Thailand, Cambodia, Loas, Vietnam
3. AQUILARIA MALACCENSIS, found in Thailand, India, Indonesia
4. AQUILARIA APICULATA, found in Philippines
5. AQUILARIA BAILLONIL, found in Thailand, Combodia, Loas, Vietnam
6. AQUILARIA BANEONSIS, found in Vietnam
7. AQUILARIA BECCARIAN, found in Indonesia
8. AQUILARIA BRACHYANTHA, found in Malaysia
9. AQUILARIA CUMINGIANA, found in Indonesia, Philippines
10. AQUILARIA FILARIA, found in Nuegini, China
11. AQUILARIA GRANDIFLORA, found in China
12. AQUILARIA HILATA, found in Indonesia, Malaysia
13. AQUILARIA KHASIANA, found in India
14. AQUILARIA MICROCAPA, found in Indonesia, Malaysia
15. AQUILARIA ROSTRATA, found in Malaysia
16. AQUILARIA SINENSIS, found in China

(Source: Ng, L.T., Chang Y.S. and Kadir, A.A., 1997)

Table 2.1: Comparison of four types of Gaharu based on agarwood oil yield and prices

	Type of gaharu	**Oil yield**	**Price**
1	SUBINTEGRA	Good	Good
2	CRASSNA	Medium	Medium
3	MALACCENSIS	Poor	Poor
4	BAILLONIL	Poor	Poor

(Source: www.gaharuonline.com/gaharu_species.htm)

Figure 2.3: Species of gaharu